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Preview



# A Role for Angiopoietin-2 in Organ-Specific Metastasis

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**Angiopoietin-2 (ANG2/ANGPT2) has recently emerged as a promising target for the inhibition of tumor metastasis. In this issue of *Cell Reports*, Minami et al. show that the calcineurin-NFAT pathway promotes pulmonary tumor metastasis by inducing ANG2 in the lung endothelium.**

In order to colonize distant organs and form metastases, circulating cancer cells need to overcome the physical barrier represented by the continuous endothelial cell (EC) layer of the blood vessels in the target organ. This process, termed "extravasation," may be facilitated by factors—either secreted by the primary tumor or induced locally at the metastatic site—that enhance the permeability of the blood vessels in the target organ and favor the transmigration of the cancer cells to the new environment (Valastyan and Weinberg, 2011). In this issue of *Cell Reports*, Minami et al. (2013) show that tumor-derived vascular endothelial growth factor (VEGF) activates a calcineurin-NFAT pathway that increases *Ang2*/*Angpt2* transcription preferentially in the lung ECs in order to facilitate the pulmonary metastasis of cancer cells (Figure 1). These findings uncover a mechanism linking tumor-derived VEGF to ANG2-induced metastasis.

ANG2 is a proangiogenic factor secreted by activated ECs that binds the TIE2 receptor in a cell-autonomous fashion in order to destabilize the blood vessel and sustain angiogenesis (Gerald et al., 2013). Recently, ANG2 was shown to also promote pulmonary metastasis in mouse models of cancer (Mazzieri et al., 2011). Although these prometastatic effects may depend, at least in part, on ANG2-induced loosening of EC junctions and increased vascular permeability (Holopainen et al., 2012), it is currently unclear whether they are primarily mediated by ANG2 secreted remotely by the primary tumor or involve the local induction of ANG2 in the metastatic niche.

The NFAT proteins are transcription factors that regulate diverse cellular functions. Upon the release of calcium from intracellular compartments, calcineurin dephosphorylates NFAT proteins and triggers their translocation to the cell nucleus. In ECs, VEGF signaling increases intracellular calcium levels, thereby activating NFAT-mediated transcription of target genes (Mancini and Toker, 2009). Although it has long been known that VEGF signaling activates *Ang2* transcription in ECs (Oh et al., 1999), the molecular regulation of this response is currently poorly understood.

Minami et al. (2013) have identified NFAT binding sites in the *Ang2* enhancer and showed VEGF-induced activation of a reporter construct containing these sequences. Consistent with a direct role of NFAT in *Ang2* activation, overexpressing DSCR1—a negative regulator of NFAT (Mancini and Toker, 2009)—decreased *Ang2* transcription in cultured lung ECs. Furthermore, ANG2 messenger RNA and protein levels were increased in the ECs of lung tumor nodules growing in *Dscr1* knockout mice, which have hyperactive NFAT. The authors also show that the inhibition of the calcineurin-NFAT-ANG2 pathway by either genetic overexpression of *DSCR1* in ECs or ANG neutralization (using a soluble TIE2 receptor) decreased the incidence of pulmonary metastases derived from subcutaneously growing mouse B16 melanomas or Lewis lung carcinomas. Altogether, these data illustrate the direct regulation of *Ang2* transcription by NFAT.

Interestingly, the authors also find increased endothelial expression of ANG2 in metastatic in comparison to primary tumors both in mouse- and patient-derived samples. Although this observation merits further investigation, it suggests a prominent role for ANG2 in metastasis-associated processes. Notably, the specific blockade of ANG2 in mouse tumor models apparently achieves more dramatic antitumor effects in secondary (metastatic) tumors than primary tumors (Mazzieri et al., 2011). In addition to enhancing cancer cell extravasation via loosening EC junctions (Holopainen et al., 2012), ANG2 may promote subsequent vascularization of micrometastases by acting as a classic proangiogenic growth factor. Minami et al. (2013) observed enhanced expression of VCAM-1 and E-selectin in the lung endothelium of *Dscr1* knockout tumor-bearing mice, consistent with the ability of ANG2 to upregulate adhesion molecules in activated ECs (Fiedler et al., 2006). By increasing vascular permeability and EC adhesion molecules, ANG2 also promotes the extravasation of a variety of inflammatory cells (Scholz et al., 2011), some of which can foster the proinvasive functions of cancer cells. Thus, NFAT-mediated ANG2 upregulation in the lung endothelium may favor metastasis by multiple mechanisms.

A provocative insight from the study is the lung-specific role of the NFAT-ANG2 pathway during metastatic colonization. Indeed, subcutaneous mouse melanomas or Lewis lung carcinomas upregulated ANG2 expression selectively in the lung among the organs analyzed.

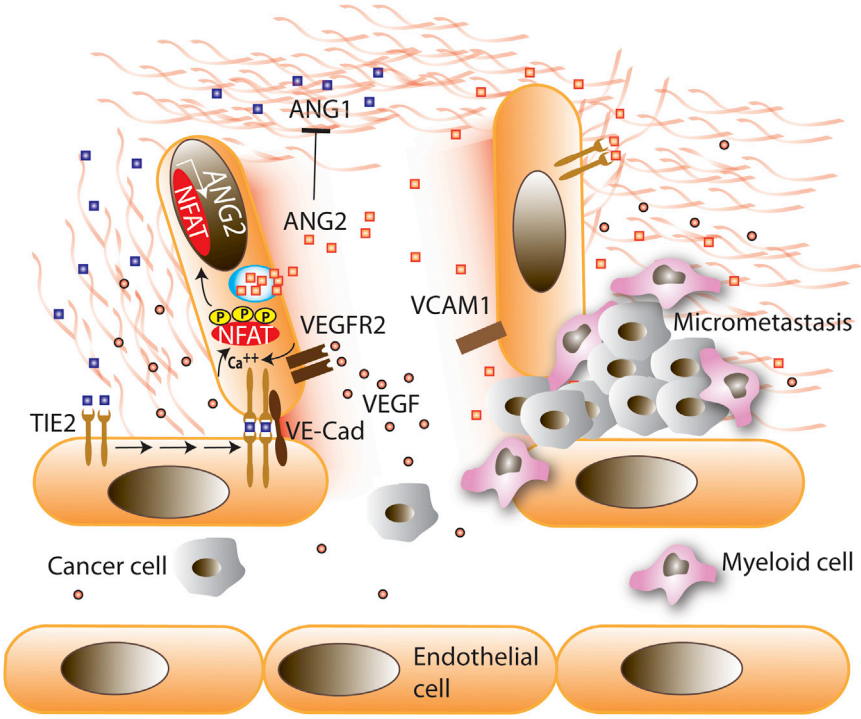


**Figure 1. VEGF-Induced Activation of the Calcineurin-NFAT Pathway Upregulates ANG2 in Lung ECs and Promotes Metastasis**

VEGFA binding to VEGFR2 induces an increase in intracellular calcium ($Ca^{2+}$), which activates NFAT-mediated *Ang2* transcription in lung ECs. Secreted ANG2 facilitates metastatic colonization of the lung parenchyma by circulating cancer cells. This process may depend on ANG2-mediated disruption of EC junctions (VE-cadherin and TIE2 complexes with ANG1) and the upregulation of EC adhesion molecules (e.g., VCAM1). Increased vascular permeability may also enhance myeloid cell extravasation. The left portion of the figure is adapted, in part, from Mancini and Toker (2009). P, phosphorylation; blue squares, ANG1; orange squares, ANG2; orange circles, VEGF.

Remarkably, it was found that preferential VEGF expression and activity in the lung could represent a key determinant of this organ-specific response. Indeed, VEGF protein levels, as well as the phosphorylation of the VEGF receptor 2, were substantially higher in the lung than in other organs harvested from tumor-bearing mice. These intriguing observations may help explain, at least in part, the preferential lung tropism of the tested tumor types. In this regard, increasing evidence indicates that specific organ microenvironments can be more or less permissive to colonization by distinct cancer cell types. Organ-specific determinants of cancer metastasis include cytokine networks and inflammatory cell types that may operate differentially in distinct organ microenvironments (Valastyan and Weinberg, 2011). The study by Minami et al. (2013) suggests a prominent role for the VEGF-NFAT-ANG2 pathway in driving the pulmonary metastasis of primary tumors and will prompt further studies that investigate the clinical significance of this phenomenon.

It is worth noting that a recent study (Im et al., 2013) showed that, although ANG2 promoted pulmonary metastasis of mouse colon adenocarcinoma cells, it limited their metastatic growth in the liver (the main site of colon carcinoma metastasis). Because organ-specific metastases might respond differently to VEGF-induced ANG2, caution should be applied to the use of ANG2 inhibitors for treating a broad spectrum of metastatic tumors. Furthermore, it remains to be seen whether additional tumor types that metastasize to the lung (e.g., breast adenocarcinomas) broadly use the VEGF-NFAT-ANG2 pathway to establish pulmonary metastasis.

The calcineurin-NFAT pathway plays important functions in immunity (Mancini and Toker, 2009). For example, it regulates cytokine production by a variety of inflammatory cells. The study by Minami et al. (2013) largely employed *Dscr1* knockout mice, which have hyperactive NFAT in most tissues. Although inflammatory cells do not express ANG2, NFAT activation in metastasis-associated inflammatory cells might have enhanced their prometastatic functions in an ANG2-independent manner. Likewise, transgenic overexpression of *Dscr1* under the control of the *Tie2* promoter—a strategy employed to suppress NFAT activation specifically in ECs—may have also affected NFAT pathway activity in subsets of hematopoietic cells, such as stem and progenitor cells and macrophages, that also express TIE2 (Mazzieri et al., 2011). Although ANG2 blockade by a soluble TIE2 receptor abrogated the effects of NFAT activation, additional studies are required in order to dissect the relative contribution of endothelial and hematopoietic NFAT activation to the observed phenotypes.

The long-term use of effective calcineurin-NFAT inhibitors (e.g., cyclosporine-A) paradoxically increases cancer risk by broadly interfering with immune cell function. Thus, targeting downstream effectors of prometastatic NFAT activation may represent a more practicable therapeutic strategy. ANG2 levels are frequently increased in the plasma of patients with cancer and often correlate with a worse prognosis (Gerald et al., 2013). By establishing a direct role for the VEGF-NFAT-ANG2 pathway in promoting pulmonary metastasis of distinct tumor types, the work of Minami et al. (2013) supports the clinical rationale of combining VEGF and ANG2 blockade for the treatment of cancer types that are prone to metastasize to the lung.

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